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THE TRANSPLACENTAL POTENTIAL DIFFERENCE AS DISTINGUISHED FROM THE MATERNAL-FETAL POTENTIAL DIFFERENCE OF THE GUINEA-PIG

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SUMMARY

1. We measured the difference in electrical potential between mother and fetus in the guinea-pig. The fetus was 33 ± 2 (S.E. of mean) mV negative with respect to the mother.

2. Salts of bromine, sulphate, rubidium, and lithium were injected into pregnant sows and blood samples were obtained from the sows and their fetuses at various times after injection. The transplacental electrical potentials at which each of these ions would be in electrochemical equilibrium across the placental exchange barrier were calculated from the Nernst equation and the concentrations in maternal and fetal plasmas.

3. The differences in transplacental electrical potential calculated from the steady-state concentrations of these ions in maternal and fetal plasmas were within 1 mV of zero.

4. These observations are consistent with a very small difference in electrical potential across the placental exchange barrier itself, and the generation of a potential difference between mother and fetus at a site other than the exchange barrier.

INTRODUCTION

A difference in electrical potential has been measured between the mother and fetus in several species. The reported values show considerable inter-species variation. The guinea-pig fetus is approximately 20 mV negative with respect to the mother (Mellor, 1969; Štulc, Rietveld, Soeteman & Versprille, 1972), the sheep fetus is about 50 mV negative (Mellor, 1970), and the goat fetus is approximately 70 mV negative (Meschia, Wolkoff & Barron, 1958; Mellor, 1970). The rat fetus is about 15 mV positive with respect to its mother (Mellor, 1969). No potential difference has been found between the mother and fetus in humans (Mellor, Cockburn, Lees & Blagden, 1969) or in rabbits (Wright, 1966; Mellor, 1969).

A potential difference across a living membrane is usually generated by the active transport of one ion or by the active exchange of two ionic species. Other ionic species are distributed across the membrane in conformance with the Nernst equation.

It has been assumed that the potential difference recorded between mother and fetus is generated at the placental exchange barrier, although the technically difficult direct measurement of the potential difference between maternal and fetal blood in the microvasculature of the placenta has never been made. If a potential difference of the magnitude commonly measured between mother and fetus was generated at the placental exchange barrier, it would be a major force affecting the passive movement of ions and charged molecules across the placenta. The electrolyte composition of fetal plasma should show evidence of such a force and knowledge of the concentrations of ions in maternal and fetal plasmas should allow one to predict the electrical potential that is governing the distribution of ions at the barrier.

Previous studies have shown the electrolyte compositions of maternal and fetal plasmas to be virtually identical in guinea-pigs (Woods, Thornburg, & Faber, 1978) and in sheep (Alexander, Nixon, Widdas & Wohlzogen, 1958; Kaiser & Cummings, 1958; Armentrout, Katz, Thornburg & Faber, 1977). If electrolytes are passively distributed then these results indicate that any potential difference across the placental exchange barrier is much smaller than the potential difference that has been measured between maternal and fetal extracellular fluids.

The present experiments were conducted in order to evaluate the transplacental potential difference by calculating equilibrium potentials for electrolytes not normally present in plasma. Salts of bromine, sulphate, rubidium, and lithium were injected into pregnant guinea-pigs and concentrations of these ions in maternal and fetal plasmas were measured at various times after injection.

METHODS

Guinea-pigs in the second half of gestation were purchased from a commercial breeder. Each sow was anaesthetized with 2% halothane in a 2:1 (v/v) mixture of nitrous oxide and oxygen, and a 1 mm o.d. polyvinyl catheter was placed in one of her carotid arteries.

After the sow had regained consciousness a saline solution containing 100 μc [^{86}Rb] rubidium chloride and 50 μc [^{35}S] sodium sulphate or 100 μc [^{82}Br] ammonium bromide (New England Nuclear, Boston, Massachusetts) was injected via the catheter. The catheter was thoroughly flushed with heparinized saline. Some of the sows received an I.P. injection of lithium chloride (1 m-equiv/kg) alone or in addition to the radiotracer. After the injection the sow was given food and water *ad libitum*.

Blood samples were obtained from the conscious sow 30 min after the injection, immediately before she was re-anaesthetized for the purpose of fetal sampling, and at appropriate intermediate times. A final maternal blood sample was also obtained after fetal blood sampling had been completed. Before each maternal sample was taken, 2 ml. blood was withdrawn from the catheter. Then a 2–3 ml. sample was withdrawn into a heparinized syringe and saved for analysis. The blood withdrawn before sampling was returned to the sow and the catheter was flushed with heparinized saline.

When the last blood sample had been taken from the conscious sow she was re-anaesthetized and her uterus was exposed through a mid line abdominal incision. A small incision was made in the uterus overlying the abdomen of a fetus. The fetal membranes and skin were grasped with a haemostat so that amniotic fluid could not leak into the fetal peritoneal cavity and a small stab wound was made through the fetal skin. Amniotic fluid which spilled on to the uterine surface was absorbed with gauze sponges in order to prevent the establishment of a short circuit across the uterine wall.

Differences in electrical potential between the mother and the *in situ* fetuses were measured with silver/silver chloride electrodes and a Keithley Model 602 electrometer which has an input impedance of 10^{14} Ω, specified by the manufacturer (it has been determined that the concen-

trations of chloride in maternal and fetal plasmas are nearly the same (Woods *et al.* 1978)). The reference electrode was placed in the maternal peritoneal cavity and the other electrode was inserted into the fetal peritoneal cavity. The asymmetry potential of the electrode pair was measured by placing both electrodes into the same saline solution before and after all electrical measurements had been made.

The uterus and fetal membranes were then opened, blood samples were withdrawn from the umbilical vein of each fetus into heparinized syringes and the times of sampling were noted. The final blood sample was then withdrawn from the mother. The fetuses and placentas were weighed.

Before analyses of the samples were made, a fetal sample was rejected if there was any question whether the uterus or placenta was perfused, as determined by inspection of the blood vessels. The blood samples were then centrifuged and the supernatant plasma was removed. The activity of ^{82}Br in a 0·5 ml. aliquot of plasma was determined by gamma spectrometry in a Packard Model 3002 scintillation spectrometer. The plasma proteins in 0·5 ml. of each sample containing ^{86}Rb and $^{35}SO_4$ were precipitated with 0·5 ml. 10 % trichloroacetic acid. The solution was thoroughly mixed and centrifuged. An aliquot of 0·5 ml. of the supernatant was dissolved in fluor (Aquasol ®, New England Nuclear) and was counted in a Packard Tricarb Model 3200 liquid scintillation spectrometer. The remainders of the plasma samples were coded and sent to the Department of Clinical Pathology for the determination of lithium concentration by atomic absorption spectrometry. Because the half life of ^{82}Br is only 36 hr, the counts per minute of samples containing bromine were corrected for the radioactive decay that occurred during the period of counting the samples. In samples containing both ^{86}Rb and ^{35}S the activity of each was determined by the channels ratio method. The activities (counts per minute per millilitre plasma) of all samples containing radioisotopes were corrected for background counts. All concentrations and activities were corrected for dilution by the heparin solution in the dead space of the sampling syringes.

RESULTS

Twenty-four sows with eighty-two fetuses were studied. Thirteen of the fetuses were excluded from the study for the following reasons: seven fetuses, including two with meconium staining, were rejected from the study because of inadequate placental perfusion, four were so small that it was not possible to obtain an adequate volume of blood from them, one was dead and one was discarded because of the possibility that the blood sample had been contaminated with amniotic fluid. The sixty-nine remaining fetuses appeared to be in good condition. The weight of the fetuses ranged from 22 to 134 g.

One sow was injected with [^{35}S] sodium sulphate alone, three received only [^{86}Rb] rubidium chloride, six were given both $^{86}Rb^+$ and $^{35}SO_4^{2-}$, four were injected with [^{82}Br] ammonium bromide, two were given lithium chloride only, three received $^{82}Br^-$ and lithium and five received $^{86}Rb^+$, $^{35}SO_4^{2-}$, and lithium. Lithium was used in addition to radiotracers only for sows with large fetuses to ensure that a sufficient volume of fetal blood would be obtained to determine the concentration of both tracers. In spite of attempts to select fetuses of adequate size by palpation of the mother's abdomen, there were three cases in which there was not enough plasma left after the removal of an aliquot for radiotracer counting to determine the plasma lithium concentration for each fetus. In these cases the remaining plasmas from the entire litter were pooled and treated as one sample.

Figs. 1 to 4 show the concentrations of these exogenous electrolytes in maternal and fetal plasmas as a function of time. In order to normalize the data, we arbitrarily set the concentration of each substance found in the maternal plasma of each sow 30 min after injection equal to one; all other maternal and fetal concentrations for that sow were expressed as ratios of the maternal concentration at 30 min.

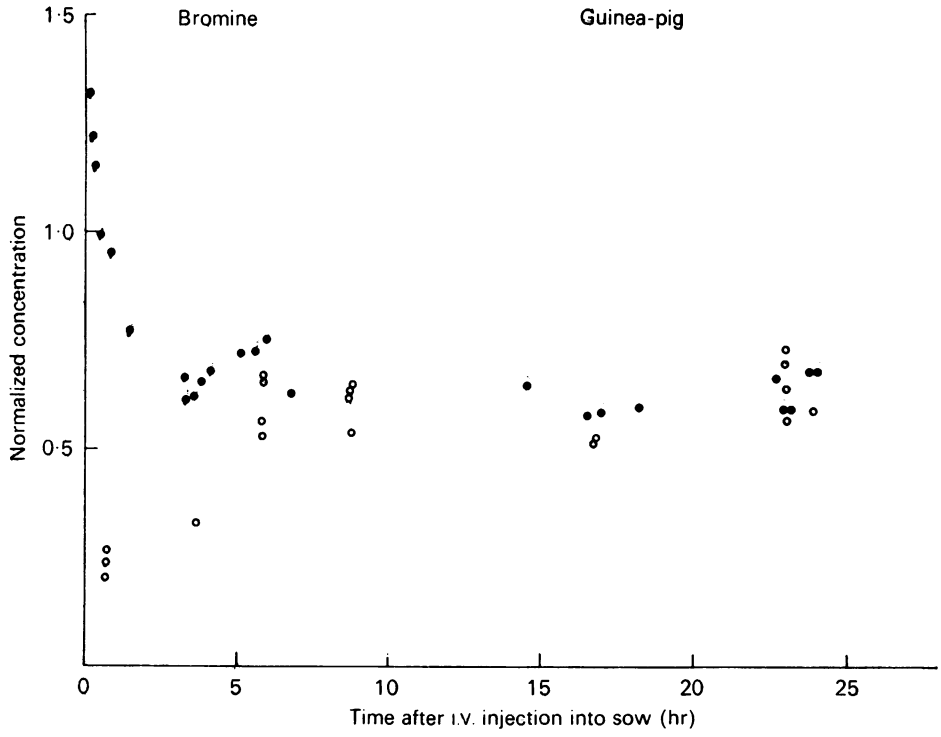

Fig. 1. Maternal (●) and fetal (○) plasma concentrations of $^{82}Br^-$ expressed as ratios of the concentration in maternal plasma 30 min after I.V. injection into the sow.

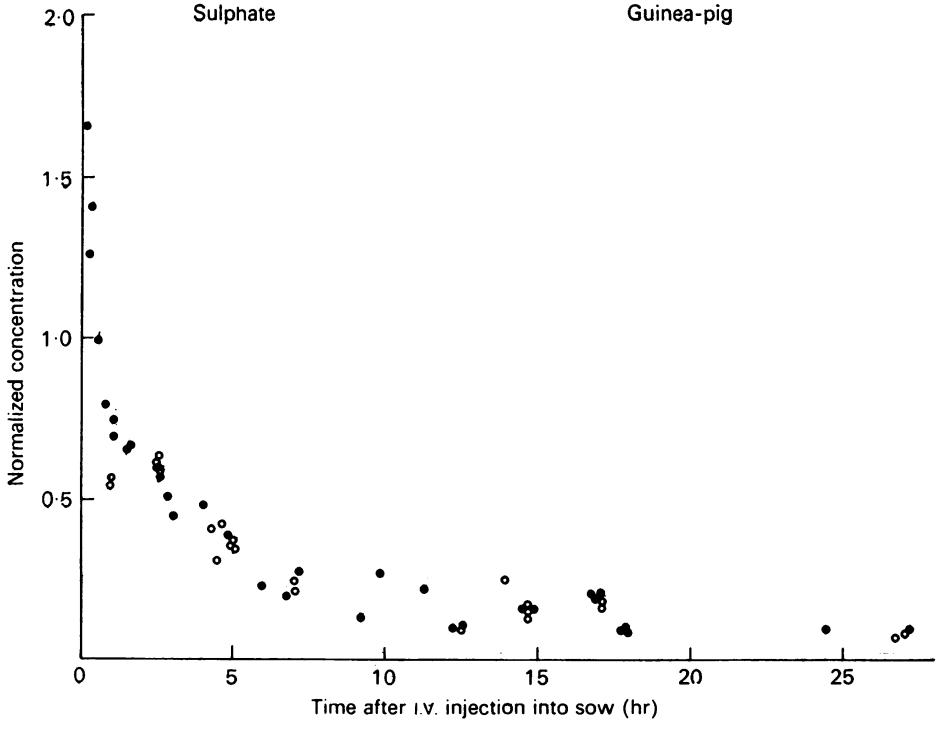

Fig. 2. Maternal (●) and fetal (○) plasma concentrations of $^{35}SO_4^{2-}$ expressed as ratios of the concentration in maternal plasma 30 min after I.V. injection into the sow.

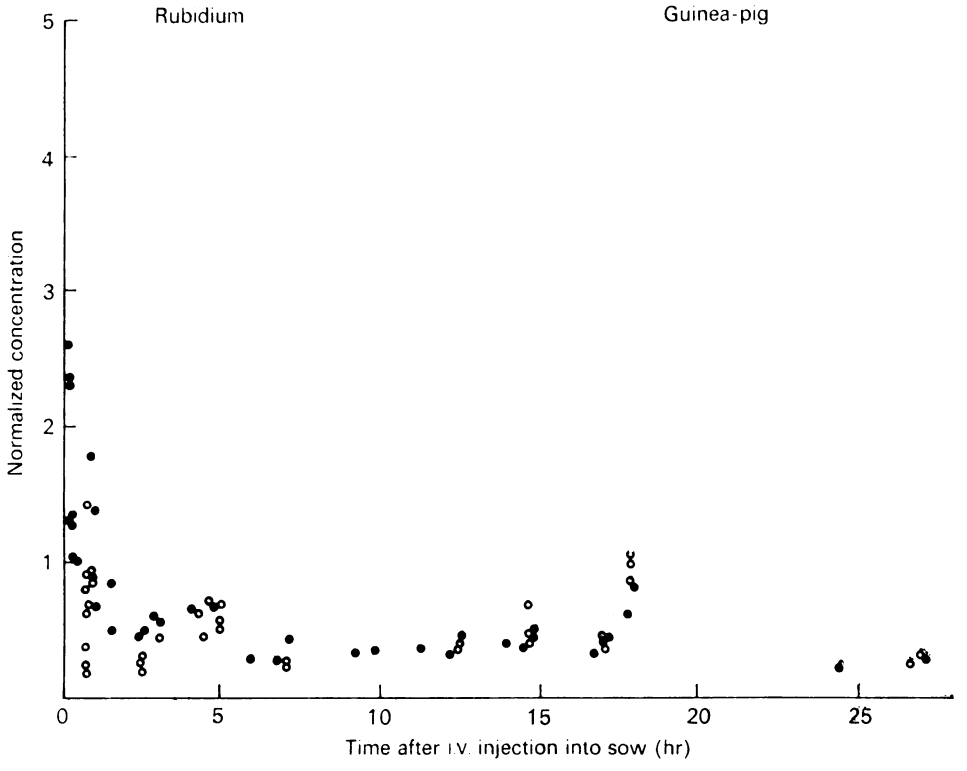

Fig. 3. Maternal (●) and fetal (○) plasma concentrations of $^{86}Rb^{+}$ expressed as ratios of the concentration in maternal plasma 30 min after I.V. injection into the sow.

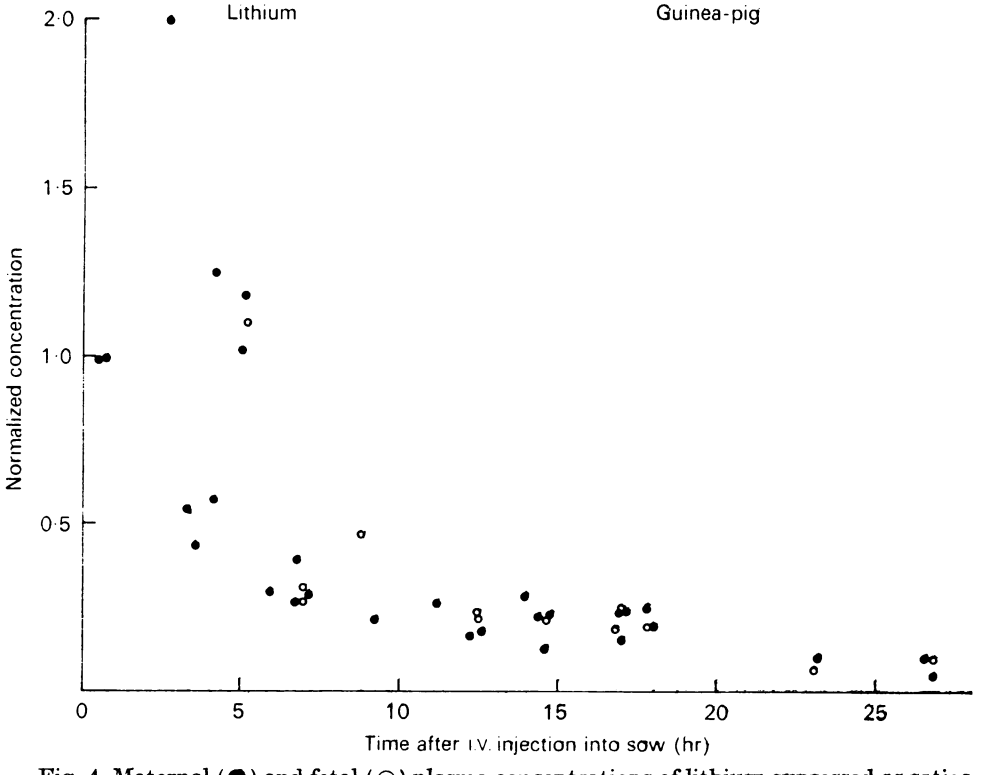

Fig. 4. Maternal (●) and fetal (○) plasma concentrations of lithium expressed as ratios of the concentration in maternal plasma 30 min after I.P. injection into the sow.

Fig. 1 shows the results for guinea-pigs that received $^{82}Br^-$. Immediately after the I.V. injection of the tracer, the maternal concentration is very high. It rapidly declines during the period of distribution to the various fluid compartments of the body. The fetal concentrations are intially low, but in 6 hr they rise to the same level as the maternal concentrations. After equilibration of the bromine in maternal and fetal plasmas there is no detectable difference in tracer concentration between the mother and the fetus.

The results for $^{35}SO_4^{2-}$ are shown in Fig. 2. In this case the maternal concentration continues to decline after the period of distribution because of excretion of the sulphate ion by the mother. The radiosulphate concentration in fetal plasma reaches the level of the maternal concentration in about 2 hr, and then declines at the same rate as the maternal concentration.

Similar results are shown for $^{86}Rb^+$ in Fig. 3. However, there is a large variability in the concentrations measured at any particular time. During the course of these experiments we observed that the concentration of rubidium in maternal plasma always rose after the sow had been anaesthetized. We surmise that the intracellular concentration of rubidium is very much higher than the extracellular concentration and that the observed rise in plasma concentration is due to a small shift of rubidium ions from their intracellular location under the influence of a slight decrease in membrane potential. Fetal samples were taken only when the animals were anaesthetized and thus it can be expected that the tracer concentration in fetal plasma would be higher than the concentration in the plasma of a different unanaesthetized sow at the same time. This systematic difference in the plasma concentrations of $^{86}Rb^+$, in addition to the normal variability in different animals, makes it more difficult to determine the exact time at which fetal concentrations have reached the same level as maternal concentrations. However, it appears certain that the concentrations of $^{86}Rb^+$ are the same in maternal and fetal plasmas 8 hr after injection.

Fig. 4 shows the results for guinea-pigs who were injected with lithium chloride. The concentration of lithium in maternal plasma rises as its is absorbed from the peritoneal cavity and then declines as it is distributed to the various fluid compartments and is excreted. No fetal blood samples were obtained before 6 hr. By that time it appears that maternal and fetal plasmas have equilibrated.

The results show a similar pattern for all of these electrolytes. The concentration in fetal plasma rises until it is essentially the same as the concentration in maternal plasma. After equilibration, changes in fetal plasma parallel changes in maternal plasma concentration. Differences in equilibration time reflect factors such as placental permeability to the ion and the volume of distribution of the ion. Changes in plasma concentration after equilibration probably reflect the rate of excretion of the electrolyte by the mother.

The difference in electrical potential between mother and fetus was measured in sixty-two maternal-fetal pairs. The mean difference in electrical potential was -33 ± 2 (S.E. of mean) mV, where the minus sign indicates that the fetus was negative with respect to the mother. There was a positive correlation between potential difference and fetal weight ($r = 0{\cdot}315$, $t = 2{\cdot}47$, $P < 0{\cdot}01$); the absolute value of the potential difference was less for larger fetuses. The order in which potential differences

were measured within a litter did not correlate with the magnitude of the potential difference ($r = 0{\cdot}106$, $t = 0{\cdot}778$, $P > 0{\cdot}1$).

If it is assumed that ions are in passive equilibrium across the placental exchange barrier, then the Nernest equation can be used to calculate the electrical potential that governs the distribution of ions across the barrier:

$$E = [RT/(zF)] \cdot \ln ([\text{ion}_M]/[\text{ion}_F])$$

E is the calculated electrical potential, R is the gas constant, T is the absolute temperature, z is the valency of the ion, F is the Faraday constant, and $[\text{ion}_M]$ and $[\text{ion}_F]$ refer to the concentrations of the ion in maternal and fetal plasmas.

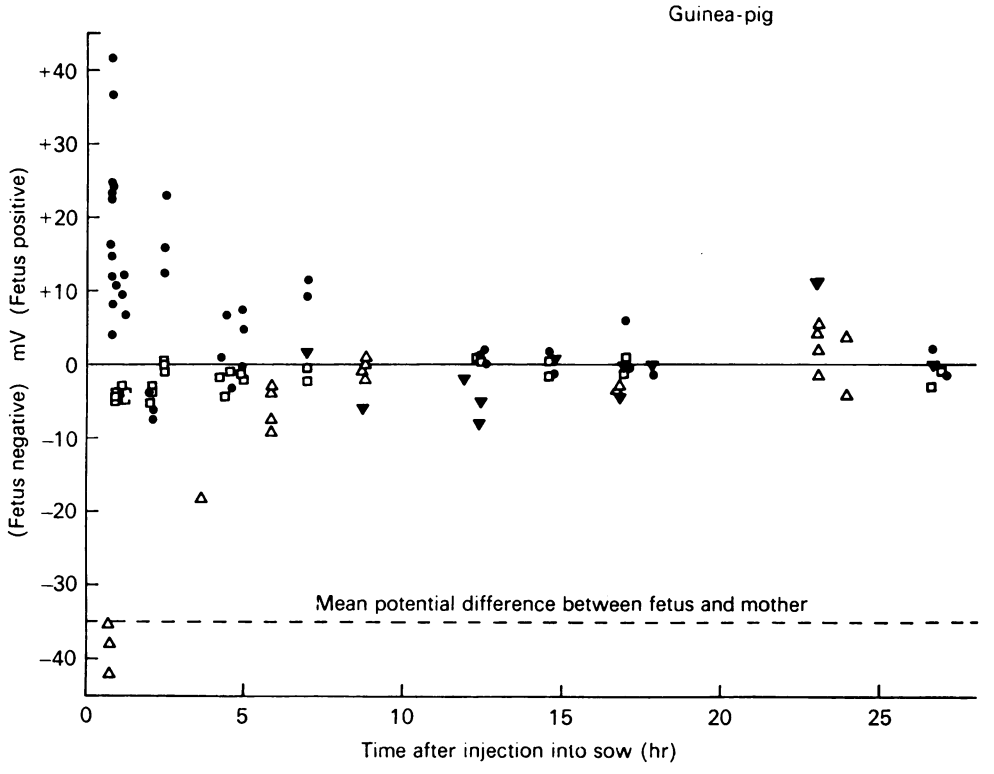

Fig. 5. Transplacental potential difference calculated from the concentrations of $^{35}SO_4^{2-}$ (□) $^{82}Br^-$ (△) $^{86}Rb^+$ (●) and lithium (▼) in maternal and fetal plasmas as a function of time after injection into the sow.

For each mother and fetus a potential difference across the placental exchange barrier was calculated from the concentrations of the tracer ion in maternal and fetal plasmas at the time of fetal blood sampling. In order to correct for changes in maternal concentration that occurred during the time between the last two maternal blood samples as well as to minimize random error, the maternal concentration at the time of fetal sampling was calculated by a linear interpolation between the concentration found in maternal plasma immediately before anaesthesia and the maternal concentration found after all fetal blood samples had been taken.

Figure 5 shows the transplacental potentials calculated from the Nernst equation

as a function of time after injection of rubidium, lithium, bromine, and sulphate ions. Initially, the calculated potential is positive for the positive ions and negative for the negative ions. This is the result expected from the effect of the polarity of the ion on the calculated potential differences when there is still diffusional transfer from the mother to the fetus. For every ion studied, the magnitude of the calculated potential decreases with time and reaches a limiting value of approximately zero at the time that equilibration between fetal and maternal plasmas occurs. The dashed line in Fig. 5 indicates the mean potential difference between mother and fetus measured during these experiments.

TABLE 1. Mean (± S.E. of mean) measured potential differences (p.d.) between mother and fetus and transplacental potential differences calculated from the equilibrium concentrations of exogenous electrolytes

Tracer	Time after injection (hr)	Calculated transplacental p.d. (mV)	Measured maternal-fetal p.d. (mV)	Fetal weight (g)
$^{35}SO_4^{2-}$	⩾ 2	−0·87 ± 0·3*	−33 ± 2*	71·7 ± 6·3
(*n*)		(21)	(19)	(21)
$^{86}Rb^+$	⩾ 8	+0·85 ± 0·7†	−34 ± 3*	87·8 ± 5·5
(*n*)		(10)	(10)	(10)
$^{82}Br^-$	⩾ 6	−0·34 ± 0·9†	−44 ± 3*	51·5 ± 10·3
(*n*)		(12)	(12)	(12)
Li^+	⩾ 7	−1·3 ± 1·1†	−37 ± 3*	57·4 ± 6·0
(*n*)		(17)	(26)	(26)

* Significantly different from zero, $P < 0·01$.
† Not significantly different from zero, $P > 0·1$.

Table 1 gives the mean calculated transplacental potential difference for each of the exogenous electrolytes used in this experiment. The means were calculated from all post-equilibration samples. The time until equilibration was determined from Figs. 1 to 4 by noting the time from which changes in fetal plasma concentration parallel changes in maternal concentration. The exact time of equilibration was not determined, but an estimate of the time at which it was reasonably certain that equilibration had occurred was made. The actual time of equilibration was probably earlier than this estimate. The mean calculated transplacental potential was not significantly different from zero for rubidium, bromine, or lithium, but was slightly different from zero for sulphate. In animals that received injections of both a positive and a negative ion there was no reliable correlation between the potentials calculated from the concentrations of the positive ion and the potentials calculated from the concentrations of the negative ion ($r = 0·268$, $t = 1·275$, $P > 0·1$). Thus it appears that slight differences in calculated equilibrium potentials can be attributed to random variation. There was no reliable correlation between the measured potential difference and the calculated potential difference for sulphate ($r = 0·229$, $t = 1·00$, $P > 0·1$), lithium ($r = -0·224$, $t = 0·91$, $P > 0·1$), bromine ($r = 0·062$, $t = 0·20$, $P > 0·5$), or rubidium ($r = -0·017$, $t = 0·15$, $P > 0·5$).

Table 2 shows the transplacental potentials calculated from the mean concentrations of electrolytes normally found in fetal and maternal plasmas of guinea-pigs (Woods

et al. 1978). The mean transplacental potential predicted from the normal concentrations of these ions is very close to zero and differs from the large electrical potential difference between fetus and mother measured in this and other experiments (Mellor, 1969; Štulc, *et al.* 1972).

TABLE 2. Transplacental potential difference (p.d.) calculated from the mean concentrations of electrolytes normally found in maternal and fetal plasmas (Woods *et al.*)

Electrolyte	Concentration maternal plasma (m-mole/kg water)	Concentration fetal plasma (m-mole/kg water)	Calculated p.d. (mV)
Na^+	142·2	140·8	+0·5
K^+	4·5	4·4	+0·6
Mg^{2+}	1·1	1·0	+1·3
Cl^-	102·7	103·3	+0·2

DISCUSSION

The results of these experiments show that if sufficient time is allowed to reach a steady state the transplacental electrical potential calculated from maternal and fetal plasma concentration of bromine, sulphate, rubidium, and lithium ions is essentially zero. This agrees with the transplacental potential calculated from the concentrations of sodium, potassium, magnesium, and chloride ions normally found in maternal and fetal plasmas. If the large electrical potential difference between mother and fetus is generated at the placental exchange barrier the concentrations of the electrolytes in the fetal plasma would have to be maintained by the expenditure of energy. It is highly unlikely that specific ionic pumps would exist for each of the ions not normally found in guinea-pig plasma or that existing pumps would have the appropriate affinity for these exogenous ions to maintain equal concentrations of them in maternal and fetal plasmas. These results are best explained by the presence of no more than a very small transplacental difference in electrical potential and the generation of a potential difference between mother and fetus at a site other than the exchange barrier.

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